

BAT Smokeless Publications

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PANEL

A. Chemistry

1. DEVELOPMENT OF A LABORATORY BASED ANALYSIS SYSTEM FOR ESTIMATION OF SNUS CONSTITUENT EXTRACTION BY USERS

Kevin G. McAdam, Ph.D., and Helena Digard, B.Sc., SRNT Conference, Saggart, Ireland, 27th-30th April 2009 (Conference Poster)

Introduction: Measuring the amount of snus constituents extracted by consumers during use contributes valuable information towards an understanding of the levels of exposure, intake and biological consequences of snus use. Conventional approaches such as clinical testing for biomarkers, or controlled user testing, comparing levels of constituents before and after use, are both associated with a number of practical limitations. These limitations include a lack of biomarkers for many constituents, and slow, expensive testing procedures. Another potential approach is the use of a routine laboratory analysis system, which could provide a robust and reproducible means of comparing different products across a range of constituents. However, as demonstrated with machine-based smoking engines, no single set of experimental conditions can reproduce the range of behaviours displayed by different users when using tobacco products. Nevertheless, for cigarette smoking, machine-based laboratory approaches remain indispensable in providing comparative information about the chemical content and potential for exposure from cigarettes. Similarly, for smokeless products such as snus, a routine laboratory based technique could offer significant benefits in terms of understanding the potential for chemical exposure and other differences between snus products.

Results: The development of this laboratory approach has been guided by human extraction data on a wide range of constituents, obtained over a typical 60-minute extraction period. A number of potential laboratory approaches have been explored. Simple immersive extraction has been shown to over-extract constituents compared to human behaviour. A review of model mouth systems highlighted the range of approaches that have been developed for the food industry, and identified a Franz cell as a plausible approach for this development. A Franz cell was physically modified to make it appropriate for snus extraction. The importance of extraction time, temperature and pressure were evaluated, and the relevance of saliva, both real and artificial, to the accuracy of extraction values is discussed.

2. IMPACT ASSESSMENT OF PROPOSED LIMITS ON US AND SWEDISH SMOKELESS TOBACCO PRODUCTS

Williamson, J., McAdam, K., and Proctor, C. SRNT Europe, Bath, UK, 6th – 9th September 2010 (Conference Poster)

Objectives: IARC Monograph 89 summarised the presence of 28 chemical agents in smokeless tobacco products (STP) including a number of tobacco specific nitrosamines (TSNAs), Benzo(a)pyrene (B(a)P), metals, volatile nitrosamines and aflatoxins. Recently two groups have proposed the establishment of toxicant content limits for STPs. The WHO Study Group on Tobacco Product Regulation (TobReg) proposed regulatory limits (dry weight basis) of 2µg/g for the combined concentration of NNN and NNK, and 5 ng/g for B(a)P. The European Smokeless Tobacco Council (ESTOC) has proposed the following toxicant limits – combined concentrations of NNK, NNN, NAB and NAT: 10µg/g; NDMA: 10ng/g; B(a)P: 20ng/g; Lead: 2µg/g; and Cadmium: 2µg/g; and sum of four specified aflatoxins: 5ng/g. This study was conducted to understand how the contents of contemporary US and Swedish smokeless tobacco products compare to these proposed limits.

Method: 70 major STPs were sampled in October 2008, consisting of 32 Swedish loose and pouched snus products and 38 US products (chewing tobacco, dry snuff, pellets, moist snuff and plug). STPs were sampled to include products from all major manufacturers. Analysis for TSNAs was undertaken by the British American Tobacco GR&D laboratory; B(a)P analysis was conducted in the GR&D laboratory and also by an independent contract laboratory; NDMA, lead and cadmium analyses were conducted by independent laboratories. Aflatoxins were not measured in this study.

Results: Under the TobReg proposals the following products failed the proposed limits – (NNN+NNK): two Snus products, half of the chewing tobacco products, and all dry snuff, moist snuff and plug products; B(a)P: one snus product, all dry snuff, moist snuff, plug and moist pellet products. Under the ESTOC proposals the following products failed the limits - TSNAs: all dry and moist snuff products; B(a)P: most dry snuff, all moist snuff and the moist pellet products; NDMA: one third to one half of dry and moist snuff products; cadmium: no products; lead: one snus and one chewing tobacco product.

Conclusions: A significant number of products fail to meet both of the proposed limits; there are differences between the two proposals but overall a greater number of products failed to meet the TobReg proposal than the ESTOC proposal (31 in contrast to 25).

3. A COMPARISON OF SELECTED VOLATILE ALDEHYDE LEVELS IN US AND SWEDISH SMOKELESS TOBACCO PRODUCTS

Faizi A.¹, Mola M.¹, Rodu. B.², McAdam K.¹ CORESTA Meeting, Aix-en-Provence, France, 18th-22nd October 2009 (Conference Presentation)

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To date, researchers have reported 28 toxicants in smokeless tobacco products (STP) including the volatile aldehydes formaldehyde, acetaldehyde and crotonaldehyde¹. According to the International Agency for Research on Cancer (IARC), formaldehyde is classified as a Group 1 carcinogen, acetaldehyde as Group 2B, and crotonaldehyde as Group 3².

Previous studies³ have shown that the contents of these volatile aldehydes in STPs ranged from 0.2ug/g to 27.4ug/g on a dry weight basis (DWB) for moist snuff, dry snuff and natural tobacco. However, approximately 20 years have passed since these measurements were carried out. Research has shown changes in levels of other toxicants in STPs over this time period⁴. Therefore, a more up to date survey was necessary to reflect current STPs on the market.

70 major STPs were sampled from Sweden and the US in October 2008. They consisted of 32 Swedish loose and pouched snus products and 38 US products including chewing tobacco, dry snuff, pellets, moist snuff and plug. The STPs were sampled to include products from all major manufacturers within Sweden and the US. The target analytes were analysed by water extraction of the STP, derivatisation to carbonyl-hydrazones and analysis by HPLC/UV. The analytical approach was also capable of analysing acrolein, itself a Group 3 carcinogen, but not on the list of 28 toxicants, and these values are also reported.

Contemporary volatile aldehydes values ranged from below detection limit (BDL) to 10.6ug/g DWB (BDL to 4.9 ug/g on a wet weight basis (WWB)) for both Swedish and US STPs.

¹ IARC Monographs on the Evaluation of Carcinogenic Risk to Humans, IARC Press, Lyon, France, Volume 89, 2007, 55-60

² www.iarc.fr

³ Brunnemann K.D. and Hoffmann D., Chemical Composition of Smokeless Tobacco Products, Smoking and Tobacco Control Monograph No. 2, 1992, 96-108

⁴ Rodu B, Jansson C., Smokeless tobacco and oral cancer: A review of the risks and determinants, Crit Rev Oral Biol Med 2004; 15: 252-263

4. A COMPARISON OF RADIOACTIVE ELEMENTS IN US AND SWEDISH SMOKELESS TOBACCO PRODUCTS

MOLA M.¹, FAIZI A.¹, RODU B.², McADAM K.¹, BENZING R.³ and PRIOR G.³ TSRC Conference, Amelia Island, Florida, USA , 17th – 20th September 2009 (Conference poster)

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A total of 28 toxicants have been reported in smokeless tobacco products (STP), including the radioactive elements ²¹⁰Po, ²³⁵U and ²³⁸U⁵. According to the International Agency for Research on Cancer (IARC), these three elements are classified as Group 1 carcinogens⁶. In contrast to the significant body of historic information available on the levels of radioactive elements in tobacco leaf and cigarettes, there is not much available information on STPs. A limited number of studies have been carried out on STPs, and activities ranging from 6 mBq/g to 74 mBq/g for snuff and natural tobacco have been reported⁷. Given the lack of comprehensive or recent data in this area, an up to date survey was conducted to reflect current STPs on the market. 70 STPs were sampled from all major manufacturers in Sweden and the US in October 2008. They consisted of 32 Swedish loose and pouched snus products and 38 US products including chewing tobacco, dry snuff, pellets, moist snuff and plug. In order to provide a more complete picture, several commonly occurring α radioisotopes (²³²Th, ²³⁰Th, ²²⁸Th, ²³⁴U, and ²²⁶Ra) and one β emitter (²¹⁰Pb) were also examined. Polonium, thorium and uranium isotopes were measured by α -spectrometry and the radium and lead isotopes were measured with a gas flow proportional counter. The combined α activity for the measured radioisotopes ranged from < 12 mBq/g to 50 mBq/g for both Swedish and US STPs, whilst the β activity of ²¹⁰Pb ranged from < 5 mBq/g to 150 mBq/g.

⁵ IARC Monographs on the Evaluation of Carcinogenic Risk to Humans, IARC Press, Lyon, France, Volume 89, 2007, 55-60

⁶ www.iarc.fr

⁷ Brunnemann K.D. and Hoffmann D., Chemical Composition of Smokeless Tobacco Products, Smoking and Tobacco Control Monograph No. 2, 1992, 96-108

5. INORGANIC TOXICANT LEVELS IN CONTEMPORARY SMOKELESS TOBACCO PRODUCTS

Kevin McAdam, Arif Faizi, Michele Mola and Brad Rodu, SRNT Conference, Baltimore, USA, 24th – 27th February 2010 (Conference poster)

Kevin G. McAdam, Ph.D., Arif Faizi, Michele Mola, Ph.D., Group Research and Development, British American Tobacco, Regents Park Road, Southampton, SO15 8TL, UK; and Brad Rodu, D.D.S., University of Louisville, 505 South Hancock Street, Louisville, KY 40202

IARC monograph 89 summarised historical literature on the presence of carcinogens in smokeless tobacco products. 28 chemical agents are listed including nitrosamines, carbonyls, benzo(a)pyrene, angelica lactones, coumarin, ethyl carbamate, and a series of metallic and radioactive species. There is significant data in the literature concerning the levels of nitrosamines in smokeless tobaccos, but there is little information available for the majority of the other species. Much of the data is 20-30 years old but smokeless tobacco product styles, ingredients and production practises have undergone significant changes over this time. Moreover, most of the existing data has been generated on a small number of brands in each study; with little comprehensive comparative information available on the contents of different product styles. A study was initiated in 2008 examining the levels of the agents in contemporary smokeless tobacco products from the US and Sweden. 70 smokeless tobacco brands, covering loose and pouched snus, chewing tobacco, dry and moist snuff, tobacco pellets and plug tobacco, were sourced covering all major manufacturers and 80-90% market share in both markets. The smokeless tobacco products were analysed for the metalloid species identified in Monograph 89 (the metals Arsenic, Nickel, Beryllium, and the radio-elements Polonium-210, Uranium-235 and -238), as well as other toxic metalloids and radioactive species previously identified in tobaccos and other plant materials (Cadmium, Chromium, Lead, Mercury, Selenium; the alpha emitters Uranium-234; Thorium-232, -230, -228; Radium-226; and beta emitter Lead-210).

The following mean values (micrograms per gram) were obtained, Nickel 1.66, Chromium 0.85, Lead 0.59, Cadmium 0.53, Selenium 0.068, Arsenic 0.03, Beryllium 0.014, and Mercury: 0.01. Mean values for the radioactive species ranged from nano-grams to atto-grams per gram. Comparison of measured values amongst different smokeless tobaccos styles, and with historic values will be presented at the meeting.

This work extends significantly the available knowledge-base on toxicant levels in contemporary US and Swedish smokeless tobaccos.

Learning Objective:

To understand and compare the levels of inorganic toxicants in contemporary smokeless tobacco products, across product styles and with historic data

Funding:

The study was funded by British American Tobacco

6. LEVELS OF NON-TOBACCO SPECIFIC NITROSAMINES IN US AND SWEDISH SMOKELESS TOBACCO PRODUCTS

McAdam, K.¹, Faizi, A.¹, Kimpton, H.¹, Williamson, J.¹, Proctor, C.¹, and Rodu, B.² SRNT Europe, Bath, UK, 6th – 9th September 2010 (Conference poster)

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Objectives: IARC Monograph 89 summarised the presence of 28 chemical agents in smokeless tobacco products (STP) including a number of nitroso species⁸ (TSNAs, nitrosoacids, and a range of non-tobacco specific nitrosamines - NTSNA). A number of NTSNA species have been identified in STPs such as (abbreviation and IARC classification): NDMA (2A), NDEA (2A), NEMA (2B), NDPA (2B), NDBA (2B), NPIP (2B), NPYR (2B), NMOR (2B), NDELA (2B), NDIPLA (3), NDIPA (not classified), and NDBzA (not classified). Research has shown changes in levels of TSNAs in STPs over the last 30 years⁹, therefore an up-to-date survey on the levels of NTSNAs was considered necessary to characterise currently available STPs.

Method: 70 STPs available on the Swedish and US markets were sampled in October 2008, consisting of 32 Swedish loose and pouched snus products and 38 US products including chewing tobacco, dry snuff, pellets, moist snuff and plug. STPs were sampled to include products from all major manufacturers. Analysis for NTSNAs was undertaken by a contract laboratory using established methods (NDMA, NEMA, NDEA, NDPA, NDBA, NPIP, NPYR) and newly developed methods (NDIPA, NMOR, NDELA, NDIPLA, and NDBzA).

Results: No NDELA or NDIPLA was detected in any of the samples tested. All of the products examined had levels of NEMA, NDEA, NDIPA, NDBA and NPIP below the limits of quantification of the methods. NDBzA was detected in four snus products but none of the other smokeless products; NDPA was quantified in 3 snus products only; NMOR was quantified in one snus and two moist snuff products only. A significant number of dry and wet snuff products had quantifiable levels of NDMA and NPYR; one snus product also had a quantifiable level of NDMA.

⁸ IARC Monographs on the Evaluation of Carcinogenic Risk to Humans, IARC Press, Lyon, France, Volume 89, 2007, 55-60

⁹ Rodu B, Jansson C., Smokeless tobacco and oral cancer: A review of the risks and determinants, Crit Rev Oral Biol Med 2004; 15: 252-263

7. ETHYL CARBAMATE LEVELS IN US AND SWEDISH SMOKELESS TOBACCO PRODUCTS

Faizi A.¹, Kimpton H.¹, Rodu B.², McAdam K.¹ CORESTA Congress, Edinburgh, Scotland, 12th – 16th September 2010 (Conference poster)

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To date, researchers have reported 28 chemical agents in smokeless tobacco products (STP) including ethyl carbamate (urethane)¹⁰. According to the International Agency for Research on Cancer (IARC), ethyl carbamate is classified as Group 2A, a probable carcinogen. Previous studies¹¹ have shown that the contents of ethyl carbamate in chewing tobacco were 310 to 380 ng/g dry weight basis (DWB). However, approximately 20 years have passed since these measurements were carried out. Research has shown changes in levels of other toxicants in STPs over this time period¹². Therefore, a more up-to-date survey on the levels of ethyl carbamate was considered necessary to reflect current STPs on the market.

70 major STPs were sampled from Sweden and the US in October 2008. They consisted of 32 Swedish loose and pouched snus products and 38 US products including chewing tobacco, dry snuff, pellets, moist snuff and plug. The STPs were sampled to include products from all major manufacturers within Sweden and the US. Analysis for ethyl carbamate was undertaken by a contract laboratory using an established method. The absence of measurable levels of ethyl carbamate in contemporary chewing tobacco contrasts with historic data³. Only moist snuff, loose snus and pouched snus contained measurable levels of ethyl carbamate. Amongst these products, a significant number were below detection limits (the “as received” wet weight basis (WWB) limit of detection was 20 ng/g). With moist snuff products the range of values measured were <20 to 688 ng/g WWB, loose snus products <20 to 37ng/g WWB, and portion snus products <20 to 84 ng/g WWB.

¹⁰ IARC Monograph 89, IARC Press, Lyon, France, 2007, 55-60

¹¹ Brunnemann K.D. and Hoffmann D., Smoking and Tobacco Control Monograph No. 2, 1992, 96-108

¹² Rodu B, Jansson C., Crit Rev Oral Biol Med 2004; 15: 252-263

8. A METHOD FOR THE DETERMINATION OF NNAL, ISO-NNAL & NNA IN SMOKELESS TOBACCO PRODUCTS

**VAN HEEMST, J.D.H.; WRIGHT, C.G.; KIMPTON, H.; and McADAM, K.G.,
CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, Austria, 9th-
13th October 2011 (Conference presentation)**

The presence of 28 compounds in smokeless tobacco products (STPs) was summarized in IARC Monograph 89, including nitroso compounds such as NNN (N-nitrosornicotine), NNK (4-(N-methylnitrosamino)-1-(3-pyridyl)-1-butanone) and NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol). Although there have been a significant number of studies that analyzed the contents of NNN and NNK in STPs, NNAL, iso-NNAL, and particularly NNA (4-(N-nitrosomethylamino)-4-(3-pyridyl)-1-butanal) have received significantly less attention.

Historically NNAL and iso-NNAL have mainly been determined using non-specific techniques such as GC-TEA, combined with extensive sample clean-up and concentration procedures that may impact on the accuracy of data. No published method for NNA in STPs is currently available. Therefore a sensitive and specific method for these three analytes was developed and validated, in which samples of smokeless tobacco products were spiked with deuterated NNAL, iso-NNAL and NNA as internal standards. After the internal standards were allowed to soak into the samples, water was added and the samples were left to fully hydrate and swell. The samples were then extracted by subsequently adding methanol, shaking vigorously and centrifuging the extract.

Aliquots of the supernatants were analyzed directly for NNA by HPLC-MS/MS, because it was shown that NNA decomposes during the clean-up of the samples. Further aliquots were acidified with 2% formic acid in order to fully protonate the analytes, after which these aliquots were cleaned-up by cation exchange SPE. The analytes were deprotonated and eluted from the cartridges with 5% ammonia in methanol, after which these eluents were analyzed for NNAL and iso-NNAL by HPLC-MS/MS.

The method was demonstrated to be fit for purpose for the quantification of NNAL, iso-NNAL and NNA in various different STPs in the concentration range for matrix samples of 31.3-625 ng/g for NNAL and iso-NNAL and 1250-12500 ng/g for NNA.

9. DETERMINATION OF SELECTED N-NITROSOAMINO ACIDS IN SMOKELESS TOBACCO PRODUCTS

**ESSEN S.A.; WRIGHT C.G.; KIMPTON H.J. and MCADAM K.G. CORESTA
Meeting Smoke Sci.-Prod. Techno Groups, Graz, Austria, 9th-13th
October 2011 (Conference poster)**

IARC Monograph 89 summarised the presence of 28 chemical agents in smokeless tobacco products (STPs) including a number of nitrosated species such as N-nitrosonornicotine (NNN), 4-(N-methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and four N-nitrosoamino acids (N-nitrososarcosine (NSAR), 3-(N-methylnitrosamino) propionic acid (MNPA), 4-((N-methylnitrosamino) butyric acid (MNBA) and Nitrosoazetidine-4-carboxylic acid (NazCA)). Several researchers have also reported the presence of other N-nitrosoamino acids in tobacco. Over the last 20-30 years a significant number of studies have characterised the contents of NNN and NNK in STPs, and research has shown changes in levels of these compounds over this time period. However, the nitrosoamino acids have received significantly less attention. Given the lack of pertinent information in this area, a survey of the levels of 11 N-nitrosoamino acids was considered of interest to more fully characterise the chemistry of currently available STPs. The analytical methods used historically for the determination of N-nitrosoamino acids were mainly based on GC-TEA analysis, which required the derivatization of N-nitrosoamino acids and often required multiple extraction procedures in order to recover all of the relevant compounds. No method suitable for all eleven N-nitrosoamino acids has been published.

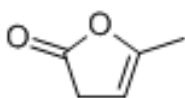
In this study an HPLC-MS/MS method was developed for the quantification of eleven N-nitrosoamino acids in a range of STPs. The compounds were extracted in water, trapped on a diatomaceous earth cartridge and separated on a polar HPLC column. Detection was performed by ESI-MS/MS. Quantification was achieved using a standard addition procedure, using deuterated analogues of three selected N-nitrosoamino acids as internal standards. In order to exclude artefactual formation of nitroso compounds during the sample preparation, a substance that does not occur naturally in tobacco was added at the beginning of the sample extraction and its nitroso analogue was monitored. The method was demonstrated to be fit for purpose for the quantification of the 11 N-nitrosoamino acids in various types of STPs.

10. DETERMINATION OF ANGELICA LACTONES IN SMOKELESS TOBACCO PRODUCTS

ESSEN S.A.; WRIGHT C.G.; KIMPTON, H.J. and MCADAM K.G.,
CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, Austria, 9th-
13th October 2011 (conference poster)

IARC Monograph 89 summarised the presence of 28 chemical agents or carcinogens in smokeless tobacco products (STPs), including α - and β angelica lactones (Figure 1). However, no information is available in the literature on their concentrations in STPs, neither are there any available analytical methods for their determination in STPs.

A) alpha-angelica lactone



B) beta-angelica lactone

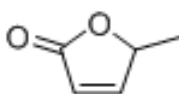


Figure 1. Structures of alpha-angelica (A) and beta-angelica (B) lactone.

In order to establish whether they are present in contemporary STPs an analytical method was developed for their analysis and quantification. The method is based on SPME extraction of the compounds from the tobacco matrix followed by HS-GC-MS analysis, using a WAX column and SIM detection. Quantification of α - angelica lactone is achieved using standard addition in combination with acetophenone- d_3 as the internal standard. The fitness for purpose of the method is demonstrated, e.g. the limit of quantification is 65 ng/g and the calibration range is 65 – 430 ng/g. The method for β - angelica lactone is only semi-quantitative, as no reference substance could be obtained for this compound. Hence, quantification is achieved using α - angelica lactone as reference and assuming a similar response. During the work it was noted that degradation of angelica lactones during sample extraction may occur in protic solvents.

11.DETERMINATION OF HYDRAZINE IN SMOKELESS TOBACCO PRODUCTS

DAVIS P., KIMPTON H., WRIGHT C. and MCADAM K.G. CORESTA
Meeting Smoke Sci.-Prod. Techno Groups, Graz, Austria, 9th-13th
October 2011 (Conference presentation)

Hoffmann and co-workers (Liu, Schmeltz & Hoffmann, Anal. Chem., 1974, 46 (7), pp 885–889) have previously reported the determination of hydrazine, which has been classified by IARC (IARC Monograph 71, 1999) as Group 2B (possibly carcinogenic to humans), in tobacco and tobacco smoke. However, no information is available in the literature on the concentrations of hydrazine in contemporary smokeless tobacco products (STPs).

In order to establish whether hydrazine is present in contemporary STPs a method was developed for its analytical measurement. Hydrazine was extracted from the STPs by agitation with methanol/0.1M hydrochloric acid. After centrifugation, an aliquot of the supernatant was derivatized using pentafluorobenzaldehyde to form decafluorobenzaldehyde azine (DFBA). The DFBA was subsequently partitioned into hexane and reduced in volume by rotary evaporation, before quantification by GC/MS using a Varian 3800/Saturn 4D instrument fitted with a 30m x 0.25mm DB5 capillary column.

The method was validated by the assessment of linearity of response, recovery, accuracy and precision. The recovery of hydrazine from five different STPs fortified at three different concentrations (0.0265 – 0.53 µg/g) ranged from 64% to 105%, and the relative standard deviation of 5 replicate analyses ranged from 3.2% to 12% across this concentration range.

12. DETERMINATION OF THE LEVELS OF NNAL, ISO-NNAL AND NNA IN CONTEMPORARY US AND SWEDISH SMOKELESS TOBACCO PRODUCTS

Kevin G. McAdam, Jasper van Heemst, Harriet Kimpton, Christopher G Wright, Justine Williamson, SRNT Conference, Toronto, Canada, 16th – 19th February 2011 (Conference poster)

*Group R&D, British American Tobacco, Regents Park Road, Southampton, SO15 8TL, UK.
and Brad Rodu, University of Louisville,*

James Graham Brown Cancer Centre, 529 South Jackson Street, Louisville KY, 40202, USA

Objectives: IARC Monograph 89 summarised the presence of 28 chemical agents in smokeless tobacco products (STPs) including a number of nitroso species such as N-nitrosornicotine (NNN), 4-(N-methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and 4-(N-methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). Over the last 20-30 years there have been a significant number of studies characterising the contents of NNN and NNK in STPs, and research has shown changes in levels of these compounds over this time period. However, the nitrosamines NNAL, 4-(N-methyl-N-nitrosamino)-4-(3-pyridyl)-1-butanol (iso-NNAL) and 4-(N-methyl-N-nitrosamino)-4-(3-pyridyl)-butanal (NNA) have received significantly less attention. Given the lack of pertinent information in this area, a survey of the levels of NNAL, iso-NNAL and NNA was considered necessary to more fully characterise the chemistry of currently available STPs.

Method: 73 STPs available on the Swedish and US markets were sampled in August 2010, consisting of 32 Swedish loose and pouched snus products and 41 US products including chewing tobacco, dry snuff, pellets, moist snuff, snus, and plug. STPs were sampled to include products from all major manufacturers. Analysis for NNAL, iso-NNAL and NNA was conducted at BAT's analytical laboratory using a method developed for this study: STPs were spiked with deuterated internal standards, hydrated and extracted with methanol. The extracts were cleaned-up using ion exchange SPE. The nitrosamine levels in the resulting samples were quantified by LC-MS/MS.

Results: Significant differences were found in the levels of the nitrosamines across different tobacco product types. NNAL and iso-NNAL were measured at levels up to 2500 ng/g of NNAL and 900 ng/g of iso-NNAL. Analysis of NNA showed this compound to be unstable, with degradation occurring both within STPs and in the SPE column. However, without SPE clean-up, NNA contents were estimated to be present at levels up to 200 ng/g.

13. DETERMINATION OF THE LEVELS OF POLYCYCLIC AROMATIC HYDROCARBONS IN US AND SWEDISH SMOKELESS TOBACCO PRODUCTS

Kevin McAdam, Arif Faizi, Christopher G Wright, Harriet Kimpton and Brad Rodu, American Chemistry Society (ACS), Boston, USA, 22nd – 26th August 2010 (Conference poster)

IARC Monograph 89 identified 28 toxicants in smokeless tobaccos, including benzo(a)pyrene. A recent study of 23 PAHs in smokeless tobacco concluded PAHs were “one of the most prevalent groups of carcinogens”. In 2010 WHO Study Group (TobReg) recommended a regulatory limit for smokeless benzo(a)pyrene of 5ng/g.

We commenced a study in 2008 quantifying toxicant levels in 70 contemporary US and Swedish smokeless tobaccos, covering eight main product styles. An experienced contract laboratory measured 21 PAHs; BAT measured benzo(a)pyrene. We examined differences in and relative proportions of PAH levels, estimated interlab consistency and implications of the TobReg recommendation.

Highest levels of individual PAHs were found with moist and dry snuff. Phenanthrene, naphthalene, fluoranthene and pyrene were present at greatest levels. Most US products sampled were above the benzo(a)pyrene limit, whereas most Swedish products were below. Interlab consistency in benzo(a)pyrene measurement was a major issue in assessing compliance with the limit.

14. EXAMINATION OF FREE BASE NICOTINE AND VOLATILE ADDITIVES IN SMOKELESS TOBACCO BY HEADSPACE SPME GCMS

Kevin. G. McAdam, Ph.D., Louise Bishop, B.Sc., Helena Digard, B.Sc., Chuan Liu, Ph.D., and John Tetteh, Ph.D.; SRNT Conference, Saggart, Ireland, 27th-30th April 2009 (Conference Poster)

Free base nicotine, and tobacco ingredients, have been cited as influencing the appeal of tobacco products. Analysis of these species in tobacco products has been a significant challenge, due to the complexities of the tobacco matrix and nicotine chemistry. A method for identification of additives on tobacco, and comparison of the levels of free-nicotine, has been developed to offer greater insight into the chemical composition of smokeless tobacco products. Solid phase microextraction (SPME) combined with GC/MS was used as this technique is highly suited to flavour analysis; SPME has also been used for estimation of free-base nicotine in cigarette smoke. The analysis was conducted by placing a snus pouch, or a pellet of loose snus, into a headspace vial. A solution of Toluene-d8 in methanol was added as an internal standard. Automated SPME analysis was conducted using a Gerstel MPS2 autosampler, connected to an Agilent 5973 GC/MS. The headspace was sampled at 30°C for 2 minutes using a pre-conditioned 100-micrometer Polydimethylsiloxane (PDMS) fibre. GC analysis was conducted in splitless mode for analysis of flavour compounds, and in a 20:1 split mode, for the analysis of major constituents such as nicotine. A DBWaxETR (Agilent) GC capillary column was used to separate the headspace components. MS peak identification was conducted using Agilent Flavour and Wiley libraries. A range of smokeless tobacco products were analysed using this methodology. Three Swedish pouched snus products, two Swedish loose snus products, one unflavoured Swedish snus tobacco blend, one Canadian snus product, two US non-smokeless products. Data analysis of flavour compounds was conducted by comparison of composition synchronised chromatograms of all the samples followed by classification based on tree dendrograms and Principal Component Analysis (PCA). Significant differences were observed between unflavoured, Swedish, US and Canadian products. The data analysis techniques highlighted commonality in the flavour profiles of the Swedish tobacco products. Nicotine levels were compared across product types and compared to tobacco pH levels.

15. CONSTITUENT LEVEL COMPARISON OF SMOKELESS TOBACCO PRODUCTS USED IN EUROPE TODAY.

Justine Williamson, Helena Digard, Eian Massey, SRNT Europe Conference, Madrid, Spain, 3rd-6th October 2007 (Conference Poster)

There is currently much debate regarding the potential use of smokeless tobacco (ST) products as part of a public health strategy for reducing the health impact of smoking. Globally there are many different forms of ST products in use today, and some forms of ST are clearly associated with increases in risks of diseases such as oral cancer. Due to differences in tobacco types used, added ingredients, manufacture process and storage methods, the chemical profiles of ST products may vary widely. The objective of this study was to measure the constituent levels of a range of ST products currently permitted by legislation and available across Europe, and compare these with levels found in snus.

In Sweden the smoking incidence in males is low (<14%) which may be associated with the widespread use of snus. Swedish snus is manufactured to an industry product standard that limits the levels of certain toxic constituents e.g. tobacco specific nitrosamines (TSNAs) which are classified as known or probable carcinogens. The health risks associated with snus have been estimated to be 90-99% lower than those of smoking^{1,2}. Each of these factors may contribute to Sweden having the lowest male incidence of smoking related disease, including oral cancer, in the developed world.

Snus is banned in the EU apart from Sweden. However, permitted ST products may contain higher levels of toxic constituents and therefore may pose an increased risk to health relative to snus. Supporters of the use of ST for tobacco harm reduction purposes recommend that a regulatory framework for such products should be implemented. This would include limits on certain toxic constituents as prescribed by the industry in Sweden.

Standard analytical techniques were used to analyse the constituents (e.g. TSNAs) of a series of manufactured and hand made ST products used by both native Europeans and immigrant populations from the Indian and African sub continents.

The results demonstrate that the toxic constituent levels of ST products used today in Europe do vary considerably, and suggest that a regulatory framework for ST products that includes limits for toxic constituents should be considered.

This study was funded by British American Tobacco.

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2. Royal College of Physicians. *Report of the Tobacco Advisory Group "Protecting Smokers, Saving Lives"* London 2002, ISBN 1 860161774

16. LEVELS OF COUMARIN IN CONTEMPORARY US AND SWEDISH SMOKELESS TOBACCO PRODUCTS

McAdam, K.¹, Kimpton, H.¹, Enos T., Williamson, J.¹, Wright, C., and Rodu, B.² SRNT Europe, Turkey, 8th – 11th September 2011 (Abstract for a Conference Poster Rejected)

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² *University of Louisville, James Graham Brown Cancer Centre, 529 South Jackson Street, Louisville KY, 40202, USA*

Objectives: IARC Monograph 89 summarised the presence of 28 chemical agents in smokeless tobacco products (STP) including Coumarin, an IARC Group 3 compound. Coumarin has been banned as a food additive in the US, and is identified in the FDA TPSAC draft initial list of Harmful or Potentially Harmful compounds in STPs. However, there is no published quantitative information available on the levels of Coumarin in contemporary STPs.

Method: 70 STPs were sampled from Sweden and the US, consisting of 32 Swedish loose and pouched snus products and 38 US products including chewing tobacco, dry snuff, pellets, moist snuff and plug, with products from all major manufacturers. Analysis for coumarin was undertaken using two methods developed by British American Tobacco. A HPLC/MS/MS method based on methanolic extraction of hydrated STPs had a lower limit of quantification of 100ng/g and was used for the analysis of samples with coumarin contents in excess of this value. A cyclohexane liquid/liquid extraction approach was used to estimate contents below 100ng/g coumarin.

Results: 30 of the 32 Swedish snus products examined, and 11/13 chewing tobacco products had coumarin levels below the limit of quantification of the methanolic extraction method. However, two snus and two chewing tobacco products had higher, quantifiable levels. All of the moist snuff products had levels in excess of 300ng/g. The highest levels measured in this study were found with some dry snuff products.

Conclusions: This work provides methodology for the analysis of coumarin in different STPs, and demonstrates that coumarin is present in all of the contemporary styles of STPs investigated.

The work was funded by British American Tobacco.

Keywords: Smokeless Tobaccos, Tobacco Constituents

B. Toxicology

17. USE OF THE EPIORAL™ TISSUE MODEL TO DETERMINE THE IRRITATION POTENTIAL OF SWEDISH SNUS

Matthews, A., Neilson, L., Dillon, D., Kidd, D., Bowen, R., Payne, R., Meredith, C. (2011)., EUROTOX, Paris, France, 28th - 31st August 2011 (Conference Poster)

Abstracts / Toxicology Letters 205S (2011) S60–S179 S167

P1349

Use of the Epioral™ tissue model to determine the irritation potential of Swedish snus

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Swedish style pouched snus comprises finely ground moist tobacco and flavourings encased within a porous pouch, commonly placed under the upper lip. Product usage could potentially span prolonged periods and exert effects on the buccal mucosa. We have used the EpiOral™ buccal mucosal model for assessing irritation potential of ingredients used within snus products. Here we describe a further application of the model to assess irritation potential of base tobacco blends used within these products. Two reference products (RS1 and RS2) were manufactured free of flavourings, matched for nicotine and humectant, but differing in levels of tobacco specific nitrosamines (TSNAs) and benzo(a)pyrene. Reference products were extracted using artificial saliva, which has been shown to have an extraction efficiency for nicotine of 68.3%. The extract was applied to the EpiOral™ model (n = 3) at 5 concentrations (1, 3.16, 10, 31.6 and 100%), for time periods of 1 and 20 h. Results showed that neither RS1 nor RS2 extracts were capable of inducing cytotoxicity (indicative of irritation potential) at any concentration or time point. Cell viability after 20 h for RS1 extracts was 97.3% and 129.5% for the 1% and 100% concentrations respectively. Cell viability after 20 h for RS2 extracts was 101.1% and 131.5% for 1% and 100% concentrations respectively. The positive control (1% SDS) reduced cell viability to 28.4% and 4.9% after 1 h and 20 h respectively. We conclude that extracts of RS1 and RS2 do not exert an irritation potential when tested in this EpiOral™ tissue model.

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18. CYTOTOXICITY AND GENOTOXICITY TESTING OF EXTRACTS OF SNUS TOBACCO

Neilson, L., Dillon, D., Pearce, H., Stone, V., Meredith, C. (2009).
European Societies of Toxicology (EUROTOX), Dresden, Germany, 13th –
16th September 2009 (Conference Poster)

Abstracts / Toxicology Letters 189S (2009) S57–S273 S71

V21

Cytotoxicity and genotoxicity testing of extracts of snus tobacco

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Whereas *in vitro* methods for cytotoxicity and genotoxicity testing of tobacco smoke (particulate phase) are defined, equivalent methods for smokeless tobacco products are in development. We investigated the ability of different solvents to extract known tobacco toxicants from American and Swedish-style snus products and performed preliminary *in vitro* assays; neutral red uptake (NRU), Ames, *in vitro* micronucleus (IVMN) and mouse lymphoma assay (MLA).

Snus was extracted in solvents (500 mg/ml; w/v equivalent) following the ISO Guideline (10993-12) for biological evaluation of insoluble medical devices. Sonication of the American-style snus extracted in DMSO did not increase the extraction efficiency of representative toxicants (B(a)P—5.23 ng/ml vs 5.39 ng/ml; nicotine—3.8 mg/ml vs 3.5 mg/ml; total TSNAs—2342 ng/ml vs 2342 ng/ml). No significant differences in cytotoxicity or genotoxicity were seen in the unsonicated or sonicated extracts.

DMSO extracts of snus tobacco were compared with aqueous extracts (saline or artificial saliva with enzyme supplements (AS)) (Chou and Hee, 1994). The aqueous solvents had similar extraction efficiencies but were less efficient than DMSO for B(a)P and total TSNAs. Higher concentrations of these solvents were compatible within the test systems, compared to DMSO.

DMSO and AS extracts gave weak activity in the Ames and IVMN. Some statistically significant increases in revertants (1–2 fold) and micronuclei (>95th percentile of the historic range) were observed with associated dose relationships. By comparison, revertant increases with a typical combusted product are ~10–20 fold for Ames. No cytotoxicity was observed in the NRU and no mutagenic activity existed in the MLA.

Reference

Chou, C.C., Hee, S.S.Q., 1994. Bioassay-driven analysis of chewing tobacco extracts.

Environmental Toxicology and Chemistry 13 (7), 1177–1186.

doi:[10.1016/j.toxlet.2009.06.212](https://doi.org/10.1016/j.toxlet.2009.06.212)

19. ASSESSMENT OF THE IRRITATION POTENTIAL OF SWEDISH SNUS INGREDIENTS USING THE EPIORAL™ TISSUE MODEL

Neilson, L., Faux, S., Hinchcliffe, S., Jai, T., Meredith, C. (2009). Society of Toxicology (SOT), Baltimore, USA, March 15-19th. The Toxicologist, Volume 108, no 1, pg 307-308 (March 2009) (Conference Poster)

1485 ASSESSMENT OF THE IRRITATION POTENTIAL OF SWEDISH SNUS INGREDIENTS USING THE EPIORAL™ TISSUE MODEL.

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Our risk assessment for ingredients used in Swedish style pouched snus differs significantly from that used for traditional combusted tobacco products. Prolonged contact of the snus pouch with the oral mucosa may allow certain ingredients to exert an irritant potential on mucosal tissue. As part of our risk assessment, we have utilized an *in vitro* screen for irritancy in order to define non-irritant levels of ingredients for use in snus products. The EpiOral™ tissue model consists of cultured human epidermal keratinocytes in a differentiated multilayer with a buccal phenotype. The irritant effects of topically applied chemicals are measured using the MTT cell viability assay. Ingredients for use in snus were tested in duplicate up to a maximum concentration of 5000 µg/ml using exposure periods of both 1h and 20h. Triton X-100 and SDS were included as positive controls and DMSO as a negative control. None of the ingredients tested at concentrations up to 5000 µg/ml affected the tissue viability at either time point. Actual tissue viabilities relative to controls were: benzyl carbinol (118.6% & 114.5%), cinnamaldehyde (99.1% & 93.3%), citronellol (124.2% & 118.4%) and menthol (121.3% & 122.7%) for 1h and 20h exposure respectively. In contrast, exposure for 1h to 1% Triton X-100 or 1% SDS gave tissue viabilities relative to controls of 40.3±11.4% and 34.4±9.2% respectively, reducing to 7.1±1.8% and 7.6±1.3% respectively after 20h exposure. A reduction of ≥25% tissue viability relative to the negative controls was used to define irritancy within our model. These data demonstrate the utility of the EpiOral™ model for assessing irritation potential to the oral mucosa. In our hands, ingredients for use in snus that may possess an irritation potential are screened using this model at an early stage in our risk assessment paradigm. This assists in defining a level of ingredient use in pouched snus that is without irritant effect.

20. USE OF AN EPIORAL™ TISSUE MODEL TO ASSESS THE IRRITATION POTENTIAL OF INGREDIENTS IN SWEDISH SNUS

Neilson, L., Faux, S.P., Hinchliffe, S.J., Jai, T.S., Kumaravel, T.S., Meredith, C., (2008) European Societies of Toxicology (EUROTOX), Rhodes, Greece, 5th – 8th October 2008 (Conference Poster)

S72 Abstracts / Toxicology Letters 180S (2008) S32–S246

R40

Use of an EpiOral™ tissue model to assess the irritation potential of ingredients in Swedish snus

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Risk assessments for ingredients used in Swedish snus differ significantly from those used for combusted tobacco products, reflecting different routes of exposure. Consumption surveys reveal that the median residence time of the snus pouch in the mouth is 60 min and many users “snus” continually during waking hours. Therefore the potential exists for ingredients to exert irritant effects on the oral mucosa. Within our risk assessment, we have utilised an *in vitro* screen for irritation potential to define levels of support for snus ingredients.

The EpiOral™ tissue model uses cultured human epidermal keratinocytes in a differentiated multilayer mimicking oral epithelium. Irritant effects of applied ingredients are measured using the MTT cell viability assay. A range of concentrations of ingredients was tested in duplicate for periods of 1 and 20 h. Triton X-100 and sodium dodecyl sulphate (SDS) were included as positive controls whilst dimethyl sulphoxide (DMSO) was included as a negative control.

Geranium oil, limonene, geraniol and linalyl acetate, when tested at maximum concentrations of 5 mg/ml, did not affect tissue viability (average viabilities relative to controls ranged from 100–119.1% for 1 h exposure and 97.3–110.3% for 20 h exposure). In contrast, exposure for 1 h to 1% Triton X-100 or SDS gave tissue viabilities relative to controls of 37.1±9.7% and 30.7%±5.6%, respectively, reducing to 6.7±1.3% and 7.5±1.4%, respectively after 20 h exposure.

These data demonstrate the utility of the EpiOral™ model for assessing irritation potential to the oral mucosa and we propose its incorporation into risk assessment paradigms for snus ingredients.

doi:[10.1016/j.toxlet.2008.06.579](https://doi.org/10.1016/j.toxlet.2008.06.579)

21. DEVELOPMENT OF AN EPIORAL IN VITRO HUMAN TISSUE MODEL FOR ORAL IRRITANCY TESTING

Delves, S. J., Faux, S. P., Jai, T. S., Kumaravel, T. S., Neilson, L. R., Meredith, C. (2008). Poster presented at the British Toxicology Society Annual Congress, Guildford, Surrey, UK, April 6-9, 2008 (Conference Poster)

The EpiOral™ tissue model (ORL-200), developed by the MatTek Corporation (Ashland, MA, USA), consists of normal human epidermal keratinocytes cultured to form a multilayered highly differentiated model of human oral epithelium analogous to that found *in vivo*. The tissues have a buccal phenotype and provide the opportunity for *in vitro* irritancy testing on human tissue. The aim of this study was to evaluate the effects of substances as potential irritants using the Methyl Thiazole Tetrazolium (MTT) viability assay in the EpiOral™ tissue model as a surrogate for irritancy testing.

The initial conditions used for the experiments were those provided by the MatTek Corporation (Kubilus *et al* 2006) with modifications. These included a range of concentrations and two time points compared to a single concentration over a range of time points. The substances were applied topically at 0.3-5000µg/ml for both 1 and 20h in duplicate. Triton X-100 (1%; 10000µg/ml) and sodium dodecyl sulphate (SDS, 1%; 10000µg/ml) were included as positive controls and dimethyl sulphoxide (0.5%) was used as a negative control at both exposure periods. MTT conversion to formazan, as a measure of tissue viability, was assessed by extraction of formazan from the tissues and measuring OD₅₇₀ according to Mossman (1983). Relative toxicities were calculated as IC₅₀ values.

The results showed that following a 1h exposure to 1% Triton X-100 and SDS, tissue viabilities of 29.5±7.5% (n=6) and 27.6±2.6% (n=8) respectively, relative to control were observed. 20h exposure to the positive controls reduced the tissue viability to 6.6±1.8% (n=7) for Triton X-100 and 7.2±1.5% (n=7) for SDS. The negative control mean OD₅₇₀ values (maximum MTT conversion) within experiments showed consistency of 1.67±0.1 (n=10) for 1h and 1.68±0.2 (n=9) for 20h. 1% Triton X-100 produced an IC₅₀ of 0.77% (average of 3 replicates) for the 1h exposure. The table shows results for the tested compounds:

Test Item	Maximum Concentration Tested (µg/ml)	Average Tissue Viability at Max Concentration Tested Relative to Controls (%)		Irritant Yes/No	
		20h Exposure	1h Exposure	20h	1h
Cinnamaldehyde	5000	93.3	99.1	No	No
Eugenol	5000	81.8	86.6	No	No
Geranium Oil	5000	100.8	100.3	No	No
Phenyl Acetic Acid	5000	108.0	113.8	No	No
Bergamot Oil	1000	97.9	92.1	No	No

The MTT tissue viability assay, when applied to the EpiOral™ tissue model, proved to be a highly reliable and reproducible method for tissue viability and irritancy testing with consistent positive and negative control data. Future work will continue with the accumulation of historical data for the current procedure and testing of a range of compounds to collate a database of relative cytotoxicities (irritancy) (IC₅₀) or maximum concentrations without an effect.

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C. Exposure Assessment

22. PHARMACOKINETICS: COMPARISON OF A CIGARETTE, LOOSE SNUS, POUCHED SNUS AND NICOTINE GUM.

DIGARD H.; PROCTOR C.; MALMQVIST U.*; RICHTER A. Nicotine CORESTA Meeting Smoke Sci.-Prod. Techno Groups, Graz, Austria, 9th-13th October 2011 (Conference Presentation)

In a consumption survey of Swedish snus users, we found that users of loose snus consumed a greater weight of tobacco/day than users of pouched snus. To investigate possible differences in the nicotine absorption kinetics following use of loose snus compared to pouched snus, we conducted a randomised cross-over pharmacokinetic study in Sweden. Six products were included in the study: 2 pouched snus products (approximately 9mg and 14mg of nicotine/portion), 2 different portion sizes of loose snus (approximately 9mg and 23mg of nicotine/portion), a cigarette (1mg ISO nicotine yield) and a nicotine gum (nicotine replacement therapy, 4mg of nicotine/piece). All the products were commercially available in Sweden at the time of study initiation (January 2010).

The 20 volunteers who completed the study were daily snus users and occasional smokers for at least 6 months prior enrolment. They attended a Clinical Research Unit in Sweden on 6 occasions to test 1 product per visit. The concentration of plasma nicotine was determined at intervals for two hours during and after product use.

All of the snus products were used for a period of 1 hour, which corresponded with the median t_{\max} . The median t_{\max} for the nicotine gum was 45 minutes (usage time: 30 minutes) and 7 minutes for the cigarette (usage time: approximately 5 minutes). Systemic nicotine exposure, based on $AUC_{0-t_{\text{last}}}$ and C_{\max} increased with increasing nicotine content for both types of snus, although increases in exposure were sub-proportional to the increases in nicotine content. All of the tobacco products gave higher systemic nicotine exposure than the nicotine gum. The nicotine exposure was similar for both of the 9mg nicotine pouched and loose snus portions with no statistically significant difference seen between these two products, suggesting that product form had no effect on the absorption kinetics of nicotine.

23. NICOTINE PHARMACOKINETICS: COMPARISON OF A CIGARETTE, LOOSE SNUS, POUCHED SNUS AND NICOTINE GUM
DIGARD H., PROCTOR C., MALMQVIST U., and RICHTER A. SRNT
Conference, Toronto, Canada, 16th-19th February 2011 (Conference Poster)

In a consumption survey of Swedish snus users, we found that users of loose snus (where a consumer takes an amount of loose tobacco from a tin and forms a ball of it before placing it in the mouth) consumed a far greater weight of tobacco each day than users of pouched snus (where a portion of snus tobacco is contained in a fleece). To investigate whether there were differences in the nicotine absorption kinetics following use of loose snus as compared to pouched snus, we have conducted a randomised cross-over pharmacokinetic study in Sweden.

Six products were included in the study: 2 pouched snus products (approximately 9mg and 14mg of nicotine per portion), 2 different portion sizes of loose snus (approximately 9mg and 23mg of nicotine per portion), a cigarette (1mg ISO nicotine yield) and a nicotine gum (an over-the-counter oral nicotine replacement therapy with 4mg of nicotine per piece). All of the products were commercially available in Sweden at the time of study initiation (January 2010).

The 20 volunteers who completed the 6 product visits were daily snus users who were also occasional smokers for at least 6 months prior to the study. They attended a Clinical Research Unit in Sweden on 6 occasions to test 1 product per visit. The concentration of plasma nicotine was determined at intervals for two hours during and after product use.

All of the snus products were used for a period of 1 hour, which corresponded with the median t_{\max} . The median t_{\max} for the nicotine gum was 45 minutes (usage time: 30 minutes) and 7 minutes for the cigarette (usage time: approximately 5 minutes). Systemic nicotine exposure, based on $AUC_{0-t_{\text{last}}}$ and C_{\max} increased with increasing nicotine content for both types of snus, although increases in exposure were sub-proportional to the increases in nicotine content. All of the tobacco products gave higher systemic nicotine exposure than the nicotine gum. The nicotine exposure was similar for both of the 9mg nicotine pouched and loose snus portions with no statistically significant difference seen between these two products, suggesting that product form had no effect on the absorption kinetics of nicotine.

24. MOUTH LEVEL EXPOSURE TIME ON NICOTINE AND TSNA EXTRACTION FROM SNUS POUCHES.

**GALE N.; DIGARD H.; McADAM K.G. CORESTA Meeting Smoke Sci.-
Prod. Techno Groups, Graz, Austria, 9th-13th October 2011
(Conference Poster)**

Measuring the amount of constituents extracted by consumers of snus during use is a valuable step in estimating exposure of snus users to tobacco constituents. A factor potentially influencing the extent of exposure is the length of time that individual consumers keep snus pouches in their mouths during use. A recent survey of snus use in Sweden established that, on average, use of pouched snus extends from approximately 30 minutes to just less than 2 hours. The objective of this study therefore was to quantify the importance of use duration on constituent transfer from snus to the consumer over this time-scale.

30 volunteer pouched snus users took part in a central location trial in Sweden, each using portions of snus over ten different duration periods (from 5 to 120 minutes), randomly ordered over 3 sessions. Used and unused portions were analysed for nicotine and tobacco-specific nitrosamines; the differences in measured quantities provided an absolute and % measure of transfer from pouch to consumer during use.

Transfer of nicotine and TSNA was found to increase with increasing usage duration, with a mean transfer of 5% after 5 minutes use increasing to about 50% after 120 minutes use. Regression analysis confirmed a statistically significant ($p < 0.001$) relationship between percentage transfer and usage duration. Analysis indicated the general operation of first order kinetics; although concentration-gradient driven Fickian diffusional processes were also supported. The observations are also consistent with recent pharmacokinetic studies (e.g. Digard *et al.*, SRNT 2011, Lunell & Lunell, 2004), which showed that t_{\max} for peak plasma nicotine concentration also appears to be linked to duration of snus use.

Exposure of snus users to tobacco constituents such as nicotine and TSNA increases significantly with the duration of use.

25. ESTIMATION OF EXPOSURE TO SNUS CONSTITUENTS AMONGST SWEDISH POUCHED AND LOOSE SNUS USERS.

**DIGARD H.; GALE N.; RICHTER A.; McADAM K.G. CORESTA Meeting
Smoke Sci.-Prod. Techno Groups, Aix-en-Provence, France, 18th-22nd
October 2009 (Conference Presentation)**

Measurement of the quantity of constituents in snus before and after use provides a flexible and convenient approach for estimating exposure to a range of tobacco constituents.

Consumer studies were conducted in Sweden as central location trials, with volunteer users of loose and pouched snus. Snus was held in the mouth for 60 minutes, consistent with average consumption times for Swedish snus users. A multi-analyte methodology was developed to quantify exposure to a range of constituents from the same snus portion. Used snus, and unused control portions were analysed for nicotine, humectants, TSNAs, nitrate, sodium and chloride ions, ammonia nitrogen, and five flavour compounds. Moisture content and pH levels were also examined. Benzo(a)pyrene, dimethyl nitrosamine and selected heavy metals were incompatible with the multi-analyte methodology due to either excessive variability, levels at or near analytical quantification thresholds, or apparent interactions/ interferences with saliva in validation tests.

Similar quantities of constituents were found to be extracted from loose and pouched snus portions, although differences in the initial content levels resulted in different percentage extractions. Greater levels of variability in the results were observed with loose snus, in comparison to pouched snus, due at least in part to greater variability in portion weight. Nicotine extraction at 23.8% (loose) and 33.3% (pouched), and TSNA extraction at 24.2-26.0% (loose) and 34.6-37.9% (pouched), were both consistent with the limited amount of previously reported work. These results also provide some indications of potential mechanisms underlying constituent extraction by users, such as constituent solubility and accessibility of the constituents to saliva.

26. ANALYSIS OF TOBACCO CONSTITUENT EXTRACTION BY SNUS USERS.

Kevin McAdam, Helena Digard, Justine Williamson, and Christopher Proctor. SRNT Europe Conference, Rome, Italy, 23rd-26th September 2008 (Conference Poster)

Introduction: A better understanding of tobacco constituent exposure during snus use can provide valuable insights into its potential effects. A variety of techniques have been employed to assess exposure, from biomarkers for NNK¹ and nicotine², to the use of in-vitro extraction systems to examine toxic metals³. These approaches are restricted by either the limited range of available biomarkers, or difficulties in ensuring that in-vitro systems mimic real-world use. A versatile alternative approach is chemical analysis of the snus pouch before and after use² to determine the level of constituent extraction.

Methods: A number of “multi-analyte” methods were developed to examine extraction of multiple constituents from the same snus pouch. Extraction of constituents has been measured through “Central-Location” trials in Sweden involving volunteer snus users; snus pouches were held in-mouth for 65 minutes, consistent with consumption data reported previously⁴. “Control” pouches were sampled at the same time from the same container. Post-trial, snus pouches were stored individually, frozen and transported to British American Tobacco’s Southampton laboratories for analysis. The chemical content of extracted, used pouches was measured, and compared with levels of constituents in the unused control samples; the difference giving the amount extracted from snus by the user.

Results: Pilot studies have established significant differences in pouch moisture and nicotine content between unused and extracted pouches. On average, pouch moisture increased from 48 to 63%, primarily due to saliva absorption by the snus pouch. Nicotine levels fell by ~40%, consistent with earlier work². Measurements of a range of constituents indicated that the water solubility of a constituent is a strong driver of its extraction during snus use.

Conclusions: These multi-analyte analytical techniques provide a flexible and practical approach to the estimation of constituent extraction by snus users. Some indications of important mechanisms driving extraction have been provided by this study.

The study was funded by British American Tobacco.

1. S.S. Hecht et al. *Cancer Epidemiology, Biomarkers & Prevention* 16, (2007), 1567-1572
2. E. Lunell, and M. Lunell, *Nicotine and Tobacco Research*, 7, (2005), 397-403
3. R.S. Pappas, et al., *Journal of Analytical Toxicology*, 32, (2008), 281- 291
4. C. Proctor, et al., *Poster presentation POS1-41 SRNT 14th Annual Meeting, Portland, Oregon, (2008).*

27. INFLUENCE OF USAGE TIME ON EXPOSURE OF SNUS USERS TO NICOTINE, NNN AND NNK FROM SNUS POUCHES

Nathan Gale, Helena Digard and Kevin McAdam, SRNT, Houston, Texas, USA, 13th – 16th March 2012 (Due to present a Conference Poster)

Objectives: Measuring the amount of constituents extracted by consumers of snus during use is a valuable step in estimating exposure of snus users to tobacco constituents. A factor potentially influencing the extent of exposure is the length of time that individual consumers keep snus pouches in their mouths during use. A recent survey of snus use in Sweden established that, on average, use of pouched snus extends from approximately 30 minutes to just less than 2 hours. The objective of this study therefore was to quantify the importance of use duration on constituent transfer from snus to the consumer over this time-scale.

Method: 30 volunteer pouched snus users took part in a central location trial in Sweden, each using portions of snus over ten different duration periods (from 5 to 120 minutes), randomly ordered over 3 sessions. Used and unused portions were analysed for nicotine and tobacco-specific nitrosamines; the differences in measured quantities provided an absolute and % measure of transfer from pouch to consumer during use.

Results: Transfer of nicotine, NNN and NNK was found to increase with increasing usage duration, with a mean transfer of 5% after 5 minutes use increasing to about 50% after 120 minutes use. Regression analysis confirmed a statistically significant ($p < 0.001$) relationship between percentage transfer and usage duration. Analysis indicated the general operation of first order kinetics.. The observations are also consistent with recent pharmacokinetic studies (e.g. Digard et al., SRNT 2011, Lunell & Lunell, 2004), which showed that t_{\max} for peak plasma nicotine concentration also appears to be linked to duration of snus use.

Conclusion: Exposure of snus users to tobacco constituents such as nicotine, NNN and NNK increases significantly with the duration of use.

The work was funded by British American Tobacco.

D. Consumer Behaviour

28. PATTERNS AND BEHAVIORS OF SNUS CONSUMPTION IN SWEDEN

Helena Digard , Graham Errington , Audrey Richter , & Kevin McAdam
Nicotine & Tobacco Research, Volume 11, Number 10 (October 2009)
1175–1181 (Paper)

Introduction: Snus is an oral snuff consisting of moist finely ground tobacco which is available in a loose form or with portions of the tobacco sealed in small sachets termed “ pouches. ” The product has a long history of use in Sweden. Currently, there is very little published information on levels of consumption and usage behaviors for snus in Sweden. The objective of this study was to obtain data on the frequency and duration of loose and pouched snus consumption in Sweden and investigate usage behaviors.

Methods: Telephone surveys of snus users randomly selected from telephone directories in all regions of Sweden were conducted in 2007 and 2008. In total, 2,914 respondents answered questions on snus usage, including the types of products used and the quantity and frequency of use.

Results: The majority of respondents (96%) used either pouched or loose snus alone. A minority (12.6%) reported dual use of smokeless and combustible tobacco products. Average daily consumption was 11 – 12 g for pouched snus and 29 – 32 g for loose snus. The typical duration of use of each pouch/portion was 60 – 70 min.

Discussion: This survey has provided new insights into contemporary snus use in Sweden, such as the marked differences in daily consumption between loose and pouched snus, length of time that snus users typically keep pouches in the mouth, differential patterns of use in males and females, and the simultaneous use of multiple pouches in a small proportion of users.

29.A COMPARISON OF SNUS CONSUMPTION IN SWEDEN AND NORWAY

Proctor, C., Digard, H., Errington, G. Richter, A and McAdam, K., SRNT Europe, Bath, UK 6th – 9th September 2010 (Conference Poster)

Objectives: Sweden is an established market for snus, an oral tobacco product associated with substantially lower health risks than cigarette smoking¹, while Norway is a maturing market. Daily snus consumption data is available for snus users in Sweden², but not for Norway.

Method: We conducted an internet survey of 1663 snus users in Norway in the second quarter of 2009 which addressed consumption per day, usage factors and behaviours.

Results: Of the 1663 snus users, 727 were daily users. Of these, 31% were loose snus users, 49% were pouched users, with the remaining 20% using both pouched and loose snus. Comparative data for Sweden were: 38% loose snus, 59% pouched snus, 3% both². Males comprised 85% of the daily snus users in Norway compared to 88% in Sweden. The majority of female daily users in Norway (88%) were pouched snus users compared to 42% of males in comparison to 93% females and 54% males in Sweden. Average daily consumption in g/day was calculated by two methods; self-reported number of portions consumed per day and number of snus tins consumed per week. Both methods gave similar calculated consumptions (g/day). The average consumption of loose snus users in Norway (approx. 22g/day) was twice that of pouched users (approx. 11g/day). The average daily consumption for daily pouched snus users was similar to that reported by Swedish pouched users (11g/day vs 12g/day)². However the average consumption of daily loose snus users was lower in Norway (22g/day vs 30g/day). The observed difference in the quantity of snus consumed daily for loose users compared to pouched users in Norway was primarily due to a larger portion size for loose snus users, as the number of portions of snus consumed on average per day by the loose users and pouched users were similar (10.6 portions vs 10.8 portions, respectively), as also observed in Sweden².

Conclusion: Despite the different histories of snus use in Sweden and Norway, the consumption patterns in the two countries appeared to be similar although combined use of loose and pouched snus is currently more common in Norway.

References:

1. Tobacco Advisory Group of the Royal College of Physicians. Harm reduction in nicotine addiction. London: RCP, 2007.
2. Helena Digard et al, Patterns and behaviours of snus consumption in Sweden. Nicotine & Tobacco Research. 11(10), 1175-1181, 2009

30. A COMPARISON OF CONSUMPTION BEHAVIOUR IN SWEDISH USERS OF LOOSE AND PORTION SNUS.

A. Richter, C. Proctor, H. Digard, D. Sandi, R. Hartmann, G. Errington, K. McAdam, J. Williamson, E. Massey, and C. Meredith. SRNT Conference, Saggart, Ireland, 27th-30th April 2009 (Conference Poster)

We have previously reported initial findings of a study of snus consumption behaviour in Sweden, in which a telephone survey of around 3000 snus users was conducted between March and April of 2007¹³. Snus in Sweden is principally available in two forms – one where the tobacco is loose and the consumer takes a pinch of tobacco from the tin, and one where the tobacco has been sealed in pouches and the consumer typically takes one pouch from the tin at a time. The objective of this paper is to analyse and compare data on consumption behaviours of male users of loose snus to male users of pouched snus. The male population was relatively evenly distributed between use of loose snus (41.9%) and pouched snus (54.0%). In this study the average daily consumption was considerably higher in loose snus users (mean around 30 g per day) than pouched snus users (around 12 grams per day). In our study sample there were very few loose snus users taking less than 10 grams per day, and a significant portion using 50 grams per day, as compared with pouched snus where 50% were consuming less than 10 g per day and very few subjects consumed over 25 grams per day. Frequency of use and duration for each use was similar for both loose and pouched use, and the key factor governing the increased amount of tobacco used seemed to be the fact that loose snus users take a much larger amount of tobacco for each use than is found in a pouch.

1. Proctor, C., Massey, E., Sandi, D., Digard, H., Meredith, C., Hartmann, R. (2008). A consumption survey of snus users in Sweden. Poster presented at the 14th Annual Meeting of the Society for Research on Nicotine and Tobacco, Portland, Oregon, USA, February 27 – March 1, 2008

31. LOOSE SNUS AND POUCHED SNUS CONSUMPTION BEHAVIOUR IN SWEDEN.

Helena DIGARD, Delcio Sandi and Rudi Hartmann COMPARISON OF 62nd TSRC Conference, Nashville, Tennessee, USA, 21st – 24th September 2008 (Conference Poster)

A telephone survey was conducted in March and April 2007 of approximately 3,000 Swedish snus users to investigate snus consumption behaviour. The survey addressed a range of topics from consumption per day to usage factors.

In Sweden snus is predominately sold in two forms; loose tobacco and portioned (with the tobacco in a porous pouch), 58.8% of the participants were portioned snus users, 37.7% loose and the remainder used both. Comparing the consumption in terms of the average amount in grams per day, the portioned users use on average the least (mean 11.2g, median 10g), the loose users on average use the most, between 2.5 and 3 times more per day (mean 29.5g, median 25g) and the users of both on average were similar to but lower than the loose users (mean 25.7g, median 24g).

A number of aspects of snus usage were investigated, including position of placement in the mouth, whether the product is moved during use and how the product was disposed of. The majority of both loose and portioned snus users (98.7% of loose users and 96% for portioned) place the product under their upper lip. During use 82.4% of loose users and 62.4% of portioned users stated they never move the product around in their mouth and 99.5% of loose snus users and 79.3% of portioned snus users do not swallow the product at the end of use.

32. A CONSUMPTION SURVEY OF SNUS USERS IN SWEDEN

**C. Proctor^(*), E. Massey, D. Sandi, H. Digard, C. Meredith, R. Hartman, SRNT
Conference Portland, Oregon, USA, 27th February – 1st March 2008
(Conference Poster)**

To investigate snus consumption behaviour in Sweden, a telephone survey of around 3000 snus users – 2555 males and 359 females – was conducted between March and April of 2007. The survey addressed various topics, from average consumption per day and residence time in mouth to dependence and use of snus as a cessation aid. The male population was relatively evenly distributed between use of loose snus (41.9%) and portion snus (54.0%), while the female population predominantly used portion snus (92.7%). With regard to portion snus consumption, on average male consumption was 11.8 grams per day while the female group consumed on average 8.5 grams per day. Male portion snus users consumed on average 12 pouches a day while female portion snus users consumed an average of 10 pouches. Almost 70 % of the portion snus users answered they keep the pouch in mouth for more than 35 min. Subsequently reassessment of this question showed the median residence time in the mouth is 60 min. Twenty two percent of male snus users took their first snus within 5 minutes of waking, and 67 % of males took their first snus with 30 minutes of waking. The data was similar for females. The survey also found that around 47 % of males and females had used snus as a way to stop cigarette smoking.

**33. PRESENTATION TO LIFE SCIENCE RESEARCH OFFICE EXPERT PANEL,
Bethesda, Maryland, USA, Proctor C. and Sandi D., 3rd October 2007
(Presentation)**

No abstract available. The contents of the presentation is as follows:

1. Consumer Data:
 - a. Snus Consumption Survey in Sweden
 - b. Risk perception survey in South Africa
2. Product Data:
 - a. Chemical content of various smokeless tobaccos
 - b. Storage condition and TSNAs formation over time in Swedish-style snus

Perceptions of relative harm of snus and cigarettes among South African smokers

C. Proctor, A. Payne and J. Clayton, British American Tobacco

The use of snus is generally accepted to be far less harmful than cigarette smoking, though the role of snus in harm reduction strategies remains controversial.^[1]

In May 2005, British American Tobacco South Africa initiated a test launch of snus in a limited number of outlets in Johannesburg, South Africa. Snus is unknown as a product in South Africa, and though there is a low level of use of oral tobacco the predominant form of tobacco use is cigarettes. The snus products were produced as well known cigarette brands, and a reasonable amount of press coverage carrying a message of harm reduction accompanied the launch. The products carried the mandated warning (Tobacco causes cancer), and communication materials throughout the year-long test focused on how to use the product rather than why.

Verified adult smokers, both those who tried snus and those who refused to do so, were interviewed about their perceptions of the relative levels of harm caused by snus and cigarettes at three different times. At interview soon after the test launch 19% of trialists reported that they thought snus more harmful than cigarettes, 38% thought snus equally harmful and only 10% thought snus less harmful. 32% were unsure. The percentage of trialists who thought snus was equally or more harmful than cigarettes grew from 57% at first interview, to 63% at second interview and 68% at the time of the third interview. Of adult smokers who would not try snus, 63% thought snus to be equally or more harmful than cigarettes, rising to 79% at the time of third interview.

The study suggests that, in the absence of clear communication on relative risks, adult smokers unfamiliar with snus are likely to see it as equally or more hazardous than cigarette smoking, despite the lack of smoke.

[1] N. Gray and J. Henningfield, Dissent over harm reduction for tobacco, The Lancet, 368, 899-900, 2006

Perceptions of relative harm of snus and cigarettes among South African smokers

C. Proctor, A. Payne and J. Clayton, British American Tobacco

Introduction: In May 2005 British American Tobacco South Africa began a pilot study by introducing Swedish snus in a limited number of retail outlets (241) in and around Johannesburg. Swedish snus was generally unknown in South Africa, where the predominant form of tobacco use is cigarette smoking. The pilot study was accompanied with media announcements, which included statements that snus use was less harmful than cigarette smoking. The snus was produced as brands Peter Stuyvesant and Lucky Strike, and was sold refrigerated in branded refrigerators. At launch South African regulations required cans of snus to carry the health warning "Tobacco causes cancer". The pilot lasted around a year with relatively little communication on harm reduction following the launch. This paper reports results of responses of snus trialists and non-trialists in face to face interviews.

Methodology: Three waves of quantitative interview data was collected 8 weeks (wave one), 32 weeks (wave two) and 56 weeks (wave three) following the start of the pilot study. The study interviewed people who had tried the snus and people who had visited the point of sale but had decided not to try snus. All interviewees were cigarette smokers. A total of 270 interviews were collected from 125 different venues. Interviewees were stratified over different parts of the day, and each wave adopted a similar pattern of data collection. We report on answers to the question "In your opinion how harmful is Snus when compared to cigarettes? Is it more harmful, equally harmful or less harmful than cigarettes?"

Results: The results from people who had tried snus at least once are presented in table one. At the first interview, eight weeks after launch, only 10% of trialists answered that they believed that snus uses was less harmful than cigarette smoking. 38% thought it equally harmful and 19% thought it more harmful. The percentage of trialists who answered that they believed snus to be less harmful than cigarette smoking remained around 10% throughout the three waves of interview. However, the percentage of those who answered that that believed than snus was equally or more harmful than cigarette smoking increased through the study, from 57% 8 weeks after launch to 68% 56 weeks after launch. Non-trialists were more likely to answer that snus use was equally or more hazardous than cigarette smoking in every wave of research, but this group also showed a pattern of increasingly believing that snus was equally harmful or more harmful to cigarette smoking, with a level of 79% believing this in the last wave of the study (table 2).

Table 1 – interviews with trialists

Snus v cigarette	1 st Wave	2 nd Wave	3 rd Wave
More Harmful	19%	33%	25%
Equally Harmful	38%	30%	43%
Equally or More Harmful	57%	63%	68%
Less Harmful	10%	10%	11%
Not sure	32%	28%	18%

Table 2 – interviews with non-trialists

Snus v cigarettes	1 st wave	2 nd wave	3 rd wave
Equally or more harmful	63%	70%	79%

Conclusions: This study suggests that in a population where snus is unknown, and in the absence of a clear message of the relative risks of snus use and cigarette smoking, many smokers may assume that snus is equally harmful or more harmful than cigarette smoking. It cannot be assumed that just because snus is smokeless that smokers will consider it to be less hazardous than cigarette smoking. Smokers who would not try snus were more likely to believe that snus is equally harmful or more harmful than cigarette smoking. While this is untested in the study, it is possible that a major barrier to trial is the belief than snus use is as risky as smoking. BAT South Africa has continued beyond this first year trial. Government regulations have allowed the health warning on the front of snus cans to change from "Causes cancer" to "Tobacco is addictive".

In addition, consumer information materials produced by BAT South Africa that focused on how to use snus was changed to include statements that snus use is less hazardous than cigarette smoking. A recent survey found that among smokers trying snus, the percentage of those who thought snus equally harmful or more harmful than cigarette smoking had dropped to 41%. The role of snus in any tobacco harm reduction effort remains controversial. This study suggests that in countries where snus is unknown, considerable effort may be required to inform smokers of the relative risks of snus use compared to cigarette smoking in addition to warnings of health risks related to snus use and advice to quit.